"QbD based Approach to the Analytical Method Development and Validation for the Estimation of Some Drugs in Bulk and Pharmaceutical Dosage Form"

SYNOPSIS

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1. Introduction:-

The prevalence of diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia, continues to rise globally, necessitating the development of effective therapeutic agents.(1) Anti-diabetic drugs play a crucial role in managing this condition, and their accurate quantification in bulk and formulation is essential for ensuring therapeutic efficacy, safety, and regulatory compliance. (2)Cardiovascular disease (CVD) is the leading cause of death and disability worldwide. A dose combination therapy for the treatment of blood pressure, diuretic and anti platelet treatments has been proposed as one strategy to reduce the global burden of CVD by remarkable percentage.(3) So, developing a method for the estimation of a mixture. This research focuses on the development and validation of analytical methods for the estimation of anti-diabetic drugs and Cardiovascular drugs, specifically employing Quality by Design (QbD), High-Performance Liquid Chromatography (HPLC), and Forced Degradation studies. These methods are integral to the robust and reliable determination of drug potency and stability, which are critical for patient safety and product quality. Quality by Design (QbD) is a systematic approach to pharmaceutical development that emphasizes understanding and controlling processes to ensure predefined quality outcomes. It involves designing analytical methods that are robust, reliable, and capable of consistently delivering accurate results. QbD principles guide the development process by identifying critical quality attributes (CQAs) and critical process parameters (CPPs), ultimately enhancing method robustness and reducing variability.(4) High-Performance Liquid Chromatography (HPLC) is a widely used analytical technique in the pharmaceutical industry due to its high resolution, sensitivity, and specificity. HPLC is essential for separating, identifying, and quantifying components in complex mixtures, making it an ideal method for analyzing anti-diabetic Cardiovascular drugs in bulk and formulations. The technique involves using a liquid mobile phase to transport the sample through a column packed with a stationary phase, resulting in the separation of analytes based on their interactions with the stationary phase.(5) Forced degradation studies are critical for understanding the stability and degradation behavior of pharmaceutical compounds. These studies involve subjecting drug substances and products to various stress conditions, such as heat, light, oxidation, and hydrolysis, to induce degradation. The data obtained from for degradation studies is used to develop stability-indicating methods that can accurately detect and quantify degradation products, ensuring the stability and shelf-life of the drug.(6,7)

Background:

The accurate estimation of anti-diabetic and cardiovascular drugs in bulk and formulation is vital for ensuring the safety and efficacy of these therapeutic agents. The pharmaceutical industry relies on robust analytical methods to meet regulatory requirements and maintain high-quality standards. Traditional analytical methods, while effective, often lack the robustness and reliability needed to handle the complexities of modern drug formulations. Therefore, integrating QbD principles, HPLC, and forced degradation studies into the method development process provides a comprehensive approach to addressing these challenges.(8)

1.Quality by Design (QbD) has revolutionized pharmaceutical development by shifting the focus from empirical trial-and-error approaches to a more systematic and science-based methodology. QbD involves defining a Quality Target Product Profile (QTPP) and identifying CQAs that impact product quality .Risk assessments and Design of Experiments (DoE) are used to determine the relationship between CPPs and CQAs, ensuring the development of robust analytical methods. By understanding the sources of variability and controlling them, QbD enhances method reliability and reduces the likelihood of method failure.

Significance: Quality by Design (QbD) is a modern, scientific, risk-based approach that ensures the quality of pharmaceuticals by designing efficient and reliable processes. It shifts the focus from end-product testing to building quality into the development process. The significance of QbD lies in its ability to systematically design analytical methods that are robust and reliable, thereby minimizing variability and ensuring consistent performance.(9)

Role:

- Setting up the Quality Target Product Profile(QTPP)
- Identifying Critical Quality Attributes(CQAs)
- Design of Experiments (DoE)
- Risk Assessment: Continuous Improvement

Importance:

- Robustness and Reliability:
- Regulatory Compliance
- Cost Efficiency
- Product Quality

A] High-Performance Liquid Chromatography (HPLC) remains the gold standard for pharmaceutical analysis due to its versatility and precision. HPLC can separate complex mixtures into individual components, allowing for the accurate quantification of active pharmaceutical ingredients (APIs) and their related substances. The technique's sensitivity enables the detection of trace impurities, which is crucial for ensuring product safety. Additionally, HPLC's adaptability allows for the development of various methods tailored to specific analytical needs, such as isocratic or gradient elution, reverse-phase or normal-phase chromatography, and various detection techniques (e.g., UV, fluorescence, and mass spectrometry).

Significance: HPLC is a pivotal analytical technique in the pharmaceutical filed due to its higher solution, sensitivity, and specificity. It is the gold standard for the separation, identification, and quantification of compounds in complex mixtures. HPLC's significance is underscored by its versatility and ability to handle a wide range of analytes. (10)

Role:

- ❖ Separation and Quantification
- ❖ Method Development
- ❖ Detection and Sensitivity
- ❖ Stability Testing

Importance

- ❖ Precision and Accuracy
- ❖ Versatility
- ❖ Reproducibility
- ❖ Regulatory Acceptance

2.Forced degradation studies: are essential for understanding the degradation pathways and intrinsic stability of pharmaceutical compounds. These studies provide insights into the drug's behavior under various stress conditions, helping to identify potential degradation products and ensuring the development of stability-indicating methods. By simulating extreme conditions, forced degradation studies help establish the drug's shelf-life and storage conditions, ensuring that the drug remains effective and safe throughout its intended shelf-life.

Significance: Forced Degradation studies, also known as stress testing, and are critical for understanding the stability and degradation behavior of pharmaceutical compounds. These studies provide insights into the chemical behavior of drugs under various stress conditions, helping to identify degradation pathways and products.(11)

Role:

- **Stability Indication**: Developing stability-indicating methods that can accurately detect and quantify degradation products.
- **Degradation Pathways**: Identifying the pathways and mechanisms of drug degradation under different stress conditions (e.g., heat, light, oxidation, and hydrolysis).
- **Shelf-Life Determination**: Providing data to establish the shelf-life and storage conditions for pharmaceutical products.

Importance:

- Ensure Drug Safety
- Inform Stability Profiles
- Enhance Product Quality

Challenges and considerations:

Challenges:-

A) Quality by Design (QbD)

Complexity in Parameter Identification: Defining Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs) specific to drug requires a deep understanding of their chemical properties and interactions.

Integration of QbD Principles: Implementing QbD principles effectively in method development demands significant resources, time, and expertise to ensure systematic optimization and robustness.

Risk Assessment: Conducting comprehensive risk assessments to identify and prioritize CQAs and CPPs that impact the performance of analytical methods for these specific anti-diabetic and cardiovascular drugs can be challenging.

RP-HPLC (Reverse Phase High-Performance Liquid Chromatography)

Method Optimization: Optimizing RP-HPLC conditions (e.g., mobile phase composition, column selection, detection wavelength) to achieve baseline separation and accurate quantification of selected drugs in complex matrices such as bulk and formulation samples.

Selectivity and Specificity: Ensuring high selectivity and specificity to distinguish between target analytes and potential interferences from formulation excipients and degradation products possess a significant challenge.

Method Robustness: Maintaining method robustness over time and across different sample matrices, considering variations in sample composition and potential matrix effects.(12,13)

A)Forced Degradation Study

Designing Stress Conditions: Developing appropriate stress conditions (e.g., thermal, photolytic, oxidative, hydrolytic) that effectively induce degradation pathways specific to selected drug without compromising the stability of the analytical system.

Degradation Product Identification: Accurately identifying and characterizing degradation products formed during Forced Degradation studies, which may require advanced analytical techniques and expertise,.

Stability-Indicating Method Development: Developing and validating stability-indicating methods capable of detecting and quantifying both intact drug substances and their degradation products under stress conditions, ensuring method suitability for regulatory compliance.(14)

- **Considerations;**

Advanced Analytical Techniques: Leveraging advanced analytical techniques and instrumentation to address, specificity, and detection limits for selected drug.

Regulatory Compliance: Ensuring method development and validation activities adhere to current regulatory guidelines (e.g., ICH guidelines) for pharmaceutical analysis, facilitating smooth regulatory approval and commercialization.

Interdisciplinary Collaboration: Collaborating across disciplines including analytical chemistry, pharmacology, and regulatory affairs to address complex challenges and ensure comprehensive method development and validation.

Continuous Improvement: Embracing a culture of continuous improvement to refine methodologies, adopt emerging technologies, and integrate feedback from validation studies to enhance method performance and reliability.

Validation Strategy: Developing a robust validation strategy that encompasses all necessary parameters (e.g., accuracy, precision, linearity, robustness) specific to the analytical methods developed for anti-diabetic and cardiovascular drug in bulk and formulation samples.

Motivation for the study:

Motivation for study is due to the following reasons;

Firstly, it aims to enhance drug efficacy and safety by developing accurate and reliable analytical methods for the estimation of anti-diabetic and cardiovascular drugs in bulk and formulation. Secondly, the study seeks to meet stringent regulatory standards, particularly those outlined by the International Council for Harmonization (ICH) guidelines, ensuring that pharmaceutical formulations comply with international quality requirements. Additionally, the research addresses significant analytical challenges inherent in simultaneously quantifying multiple anti-diabetic and cardiovascular drugs in complex matrices. By advancing analytical methodologies such as Quality by Design (QbD) and optimizing techniques like Reverse Phase High-Performance Liquid Chromatography (RP-HPLC), the study aims to innovate and improve current practices in pharmaceutical analysis. Ultimately, the research to enhance pharmaceutical quality control measures ensuring consistency and reliability in drug manufacturing processes and contributing to the advancement of pharmaceutical science and technology.

2. Scope and Significance of study

1."Implementation of QbD Approach to the Analytical Method Development and Validation for the Estimation of Some Active Ingredients and Impurities from Anti-diabetic and Cardiovascular Drugs in Bulk and Pharmaceutical Dosage Form by HPLC"encompasses several key aspects.

2. It involves the development of robust analytical methods using Quality by Design(QbD) principles to simultaneously estimate anti-diabetic and cardiovascular drugs in bulk and formulation samples.

3. Additionally, the study focuses on optimizing Reverse Phase High- Performance Liquid Chromatography (RP-HPLC) methods for accurate quantification and separation of these drugs, ensuring high sensitivity and specificity.

4. Forced Degradation studies are conducted to establish stability- indicating methods capable of detecting and quantifying degradation products under various stress conditions.

5. Method validation according to International Council for Harmonization (ICH) guidelines ensures reliability, reproducibility, and regulatory compliance for pharmaceutical applications.

6. The application of validated methods to real-world pharmaceutical formulations demonstrates their practical utility in quality control and stability testing.(15,16)

7. The significance of this study lies in its potential to enhance drug quality and safety by providing accurate analytical methods, thereby ensuring the potency and safety of anti-diabetic cardiovascular drugs in formulations and improving patient outcomes.

8. It supports regulatory compliance by validating stability-indicating methods and ensuring adherence to stringent quality standards before market release.

9. The research contributes to advancing analytical methodologies in pharmaceutical analysis through innovations in QbD, RP-HPLC optimization, and Forced Degradation studies, benefiting the broader scientific community.

10. Improved method robustness and reliability optimize manufacturing processes, reduce batch failures, and maintain consistent product quality. Furthermore, the study fosters new knowledge and methodologies in pharmaceutical sciences, promoting further research and development in analytical chemistry and drug formulation.

By Addressing complex analytical challenges associated with multiple drug estimation in complex matrices, the research provides practical solutions applicable across the pharmaceutical industry, addressing critical industry challenges and enhancing overall efficiency. (17)

3. Review of Literature

A literature review reveals that there are no methods for the triple combination of selected drug.A few methods are reported for the same category of anti-diabetic cardiovascular drugs, as follows: A literature review reveals that there are no methods for the selected drugs.

1. Meetali M. Chaphekar and Purnima D. Hamrapurkar (2016) employed the Quality by Design (QbD)approach for the development and validation of a Reverse Phase-High Performance Liquid Chromatography (RP-HPLC)method for the analysis of Vildagliptinin bulk drug and pharmaceutical formulations. The method development utilized Design of Experiments to systematically explore critical method parameters such as Buffer pH, Organic Phase composition (% acetonitrile and methanol), and Organic Modifier (methanol). Statistical tools including Analysis of Variance (ANOVA) were applied to assess the significance and interaction effects of these factors on response variables like Retention time and tailing factor, leading to the determination of optimal chromatographic conditions. The separation was achieved using a Jasco CrestPack RP C18 column (250 × 4.6 mm, 5µm) with a mobile phase consisting of Buffer (pH 6): Acetonitrile: Methanol (70:10:20 v/v), and detection was performed with a Photo-Diode Array (PDA) detector at 210 nm. The method exhibited excellent linearity over the concentration range of 5-15 µg/ml with a correlation coefficient (R2) of 0.999. Precision and accuracy studies demonstrated %RSD values below 2%, confirming the method's reliability. Forced degradation studies underscored its stability-indicating nature. Overall, the developed RP-HPLC method proves suitable for the precise and accurate quantification of Vildagliptin in both bulk drug and pharmaceutical formulations, aligning with current regulatory standards.(24)

2. Subhashini Edla and B. Syama Sundhar (2014) developed a rapid, simple, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Metformin and Glibenclamidein their combined tablet dosage form. The separation was achieved on an Oyster BDS RP-C18 column (150mm x 4.6mm, 5 µm) with an isocratic mixture of Methanol, Acetonitrile, and Water in the ratio of 30:60:10 (v/v) at a flow rate of 1.0 ml/min, and UV detection was conducted at 228 nm.The retention times for Metformin and Glibenclamide were 3.17 minutes and 8.10minutes, respectively. The method demonstrated good linearity within the concentration ranges of 2-4.5 µg/ml for Glibenclamide and 200-450 µg/ml for Metformin, with high correlation coefficients (r^2 values). The developed method was validated according to ICH guidelines, ensuring its precision, accuracy, specificity, and reliability. This RP-HPLC method offers significant practical utility for the pharmaceutical industry, providing a reliable tool for routine quality control and therapeutic drug monitoring of combined antidiabetic drugs in fixed dosage forms.(25)

3. D. Thakur, A. Kaur, and S. Sharma (2017) developed a novel, sensitive, and rugged stability-indicating HPLC method using a fractional factorial design (FFD) for the simultaneous estimation of gliclazide and metformin hydrochloride in tablets without prior separation. The final optimized chromatographic conditions included a mobile phase composition of 60:20:20:0.5 (Methanol: Acetonitrile: Water), a pH of 7.5, a flow rate of 1.0 ml/min, and a column temperature of 25 °C, utilizing a C18 Nucleosil ODS column (250 × 4.6 mm^2 i.d., 5 μm) with UV detection at 227 nm. The method was validated according to ICH guidelines, demonstrating specificity, accuracy, and excellent linearity with R^2 values of 0.997 for gliclazide and 0.996 for metformin hydrochloride. The limits of detection (LOD) and quantification (LOQ) were found to be 0.035 and 0.080 μg/ml, and 0.40 and 0.55 μg/ml, respectively. This method offers a high degree of practical utility for the accurate estimation of both drugs in pharmaceutical dosage forms.(26)

4. Fernandes, Vaughan, Subrahmanyam, and Shabaraya (2018) introduced a straightforward and precise spectrophotometric method for simultaneous determination of Saxagliptin(SAXA) and Metformin hydrochloride (MET) in laboratory mixtures. The method relies on the absorbance ratio technique, leveraging the isobestic point observed at 227nm for normalization. SAXA was quantified at its absorbance maximum (210 nm), while MET's concentration was derived from the absorbance difference between 227 nm and 210 nm, ensuring independence from interfering components. All sample dilutions were prepared using distilled water. The linearity range for SAXA was 5-50 μg/ml and for MET was 2-16 μg/ml, demonstrating suitable sensitivity for analytical applications. Statistical validation confirmed the method's accuracy, and recovery studies substantiated reliable quantification of both drugs in mixtures. This spectrophotometric approach offers a reliable tool for routine analysis in pharmaceutical quality control, emphasizing simplicity, accuracy, and precision in simultaneous estimation of SAXA and MET.(27)

5. Raghvendra Singh Bhadauria (2019) presents a UV-Spectrophotometric simultaneous equation method for the estimation of dapagliflozin (DAPA) and saxagliptin(SAXA), aimed at providing a simple, precise, and accurate analytical approach. The method involves solving simultaneous equations based on absorbance measurements at wavelengths specific to SAXA (222 nm) and DAPA (276 nm) in phosphate buffer pH 6.8. Both drugs demonstrated linear behaviour within the concentration range of 5-25 μg/ml, adhering to Beer's law. High percentage recoveries (98.44-99.05%) underscored the method's accuracy, while precision was confirmed with relative standard deviations below 2% for both analytes. (28)

6. Mulchand A. Shende (2019) presents a reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous determination of Metformin (MET), Voglibose (VOG), and Pioglitazone (PIO) in pharmaceutical dosage forms, aiming to simplify treatment through single-tablet triple fixed-dose combinations of oral hypoglycemic agents. The chromatographic separation was

performed on a Younglin (SK) gradient System with a UV 730 D detector using a Cosmosil C18 column (250 x 4.6 mm, 5µm) maintained at 45°C. The mobile phase consisted of 0.1% v/v acetonitrile: triethylamine (30:70, v/v), pH 2.5, flowing at 0.8 ml/min, with detection at 232 nm. MET, PIO, and VOG exhibited linearity over the concentration ranges of 200-600 µg/ml, 30-90 µg/ml, and 0.08-0.24 µg/ml, respectively, with regression equations indicating good correlation (R2 > 0.998). The method demonstrated high accuracy with % RSD and recoveries ranging between 99.79-102.61% for PIO, 100.57-101.60% for MET, and 100.02-101.05% for VOG. Analysis of marketed formulations confirmed the method's reliability, with mean % amount close to 100% and % RSD values ≤ 2.0%. This RP-HPLC method, validated according to ICH guidelines, proves sensitive and selective for the simultaneous estimation of MET, VOG, and PIO in combined dosage forms, making it suitable for routine quality control analysis in pharmaceutical industries.(29)

7. Ahmed Gedawy, Hani Al-Salami, and Crispin Dass (2019) developed and validated an efficient and simple HPLC method for the simultaneous determination of gliclazide and metformin hydrochloride in bulk and marketed products. The separation was achieved on an Alltima CN column(250 mm × 4.6 mm x 5 µm) in isocratic mode, with a mobile phase consisting of 20 mMammonium formate buffer (pH 3.5) and acetonitrile in a 45:55 (v/v) ratio. The flow rate was not specified. The drug peaks were well separated and detected by a UV detector at 227nm. The method demonstrated linearity in the concentration range of 1.25–150 µg/ml for gliclazide and 2.5–150 µg/ml for metforminValidation according to ICH guidelines confirmed the method's system suitability, specificity, precision, accuracy, and robustness. The limits of detection (LOD) and quantification (LOQ) for metformin were 0.8 µg/ml and 2.45 µg/ml, respectively, while for gliclazide, the LOD and LOQ were 0.97 µg/ml and 2.95 µg/ml, respectively. This method is suitable for routine quality control analysis of gliclazide and metformin in pharmaceutical formulations.(30)

8. M. K. Munde, N. S. Kulkarni, and R. H. Khiste (2020) developed and validated four new UV spectrophotometric methods for the simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk and tablet formulations. The methods include simultaneous equation, absorbance ratio, area under the curve, and first derivative (zero crossing) spectroscopic methods. In the simultaneous equation method, absorbance was measured at 224 and 232 nm for both drugs, while in the absorbance ratio method; the same wavelengths were used for estimation. The area under the curve method involved estimating both drugs at 224 and 232 nm. The first derivative (zero crossing) method transformed UV spectra into first derivative spectra, measuring the first derivative signal at 224 nm for Empagliflozin and 232 nm for Metformin hydrochloride, using a wavelength interval (Δλ) of 2 nm and a scaling factor of 1. These methods were found to be simple, fast, highly sensitive, and cost-effective, making them suitable for the routine quality control analysis of Empagliflozin and Metformin hydrochloride in commercial tablet formulations.(31)

9. M. K. Munde, N. S. Kulkarni, A. K. Sen, and D. B. Sen (2020) developed and validated a novel stability indicating analytical method using High Performance Thin Layer Chromatography (HPTLC) with a Design of Experiment (DoE) approach for the simultaneous quantification of Metformin hydrochloride and Empagliflozin in bulk and dosage forms. The optimization process involved variables such as the proportion of Isopropyl alcohol in the mobile phase, saturation time of the chamber, and distance travelled by the mobile phase. The experiments were conducted on silica gel pre-coated plates using a mobile phase composed of 2% Ammonium acetate: Isopropyl alcohol: Triethylamine(4:6:0.1 v/v/v), with chromatograms evaluated using a TLC scanner in reflectance/absorbance mode at 242 nm. The method was validated according to ICH Q2 (R1) guidelines, demonstrating correlation coefficients of 0.985 and 0.988 for Metformin hydrochloride and Empagliflozin, respectively. Calibration curves were linear in the concentration ranges of 5000-30000 µg band-1 and 125–750 µg band-1. The method exhibited % recovery between 99.05% to 102.54% for Metformin hydrochloride and 99.20% to 101.50% for Empagliflozin.Furthermore, the method successfully determined the drugs in their degradation products under forced degradation conditions such as hydrolysis, oxidation, thermolysis, and photodegradation, highlighting its stability indicating capability. This approach offers a robust tool for routine analysis and stability studies of Metformin hydrochloride and Empagliflozin in compliance with regulatory requirements.(32)

10.Sachin Bhanudas Gurav and Neela Manish Bhatia (2020) developed and validated a novel, precise, selective, and sensitive stability-indicating RP-HPLC method for the simultaneous determination of Saxagliptin hydrochloride (SAX) and Metformin HCl (MET) in bulk drug and pharmaceutical dosage forms. The chromatographic separation was conducted on a Phenomenex Luna SCX column(250 x 4.6 mm, 10 µm) using a mobile phase consisting of ammonium dihydrogen phosphate buffer pH 2.50: methanol (70:30 % v/v). Detection was performed at 210 nm, with SAX and MET eluting at retention times of approximately 6.2 min and 10.4 min, respectively. Calibration curves demonstrated linearity over the concentration ranges of 5-100 µg/mL for SAX and 40-800 µg/mL for MET. The method successfully determined peak purity in stressed samples containing major degradation products. Validation studies confirmed the method's adherence to ICH guidelines, establishing its specificity, precision, linearity, ruggedness, and robustness.(33)

11. Balamurugan Krishnan and Kirtimaya Mishra (2020) presented a comprehensive study on the development, optimization, and validation of an isocratic RP-HPLC method for the simultaneous separation of sitagliptin and metformin in bulk and pharmaceutical formulations. The method utilized a Monolithic C18 column (100×4.6 mm, 5 µm) with PDA-UV detection at 210 nm. Optimization parameters included methanol: acetonitrile in the range of 40-50% (v/v), pH adjusted to 3.5-4.5 using diluted orthophosphoric acid, and a flow rate of 0.3-0.5 ml/min.The optimized conditions, methanol:acetonitrile (pH 3.5±0.5) with a flow rate of 0.484 ml/min and pH 3.946, enabled efficient

separation with metformin and sitagliptin eluting at retention times of 3.3 and 4.4 minutes, respectively. The method was validated according to ICH guidelines, confirming its specificity, accuracy, linearity, precision, and robustness for routine quality control analysis of pharmaceutical formulations containing sitagliptin and metformin. This study underscores the application of quality by design and multi-criteria decision-makingapproaches in method development, ensuring reliable quantification of gliptin derivatives in combination therapies for diabetes management.(34)

12. Razan A. Ahmad and Mohammad M. Hailat (2021) developed an economical, simple, precise, selective, and stability-indicating RP-HPLC method for the simultaneous determination of empagliflozin, pioglitazone, and metformin in bulk and tablet dosage forms. The method adhered to ICH guidelines and utilized a mobile phase consisting of orthophosphoric acid buffer and acetonitrile (30:70 v/v) adjusted to pH 2.7. Chromatographic separation was achieved on an ACE C18 column (250 mm x 4.6 mm, 5 µm) at a flow rate of 0.5 mL/min and a detection wavelength of 230 nm, maintaining a column temperature of 25°C. The method exhibited high linearity ($R^2 \geq 0.9998$) over the concentration range of 20-250 ppm for all three drugs. Stability studies under various stress conditions confirmed the method's suitability as a stability-indicating assay. The retention times were 3.2 min for empagliflozin, 2 min for metformin, and 2.6 min for pioglitazone, with a total runtime of 7 min. The method demonstrated robustness against variations in mobile phase pH, detector wavelength, temperature, and composition. Validation parameters including limit of detection, limit of quantification, precision (% RSD < 2%), accuracy, and recovery (%) further validated its applicability for routine analysis in pharmaceutical formulations containing empagliflozin, pioglitazone, and metformin.(35)

13. S. S. Khoja and L. J. Patel (2021) a study on the development and validation of a highly sensitive, precise, and accurate liquid chromatography with mass spectrometry (LC-MS/MS) method for the simultaneous determination of Ertugliflozin and Sitagliptin in the combined formulation of Steglujan 15 mg/100 mg film-coated tablets®. The chromatographic separation was achieved using a Phenomenex Gemini C18 column (150 × 4.6 mm, 5 µm) with an isocratic mobile phase consisting of 0.1% formic acid: acetonitrile(10:90, v/v), operated at a column temperature of 40°C and a flow rate of 0.6 mL/min. Mass spectrometric detection was performed under multiple reactions monitoring (MRM) mode with electrospray ionization, monitoring m/z 437.10-328.95 and m/z 408.10-234.95 transitions in positive mode for Ertugliflozin and Sitagliptin, respectively. The method demonstrated linearity over the concentration ranges of 15-450 µg/mL for Ertugliflozin and 100-3000 µg/mL for Sitagliptin. Validation according to International Conference on Harmonization (ICH) and FDA guidelines confirmed the method's suitability for accurate quantitation of Ertugliflozin and Sitagliptin in pharmaceutical dosage forms, ensuring its

applicability in routine quality control analysis.(36)

14. M. Attimarad and K. N. Venugopala (2021) introduced a new RP-HPLCmethod for the simultaneous determination of vildagliptin (VIL) and remogliflozin (REM) in a pharmaceutical formulation designed for treating type 2 diabetes mellitus. The study employed vildagliptin as an internal standard (IS) and utilized a Zorbax C18 column with isocratic elution of acetonitrile and phosphate buffer (pH 5) in a ratio of 55:45 v/v at a flow rate of 1.2 mL/min. Detection was carried out at a wavelength of 210 nm, with the method validated according to ICH Q2 requirements. The optimized HPLC method demonstrated satisfactory linearity over the concentration ranges of 10-60 µg/mL for VIL and 10-100 µg/mL for REM, supported by regression coefficients (R^2) ≥ 0.998. Accuracy assessments yielded average values of 99.57% for VIL and 100.59% for REM with low relative errors. Precision was confirmed by low relative standard deviations. Robustness analysis using a Pareto chart showed minimal impact of minor experimental variations on test results. Overall, the developed method proved effective in simultaneous quantification of VIL and REM from the formulation, indicating its suitability for routine quality control in pharmaceutical laboratories.(37)

15. Kapil Rana and Pushpendra Sharma (2021) developed a straightforward and precise reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous analysis of Metformin hydrochloride, Nateglinide, and Pioglitazone hydrochloridein tablet dosage forms. Chromatographic separation was achieved using a 25cm × 4.6mm i.d., 5-µm particle size Cis column with a mobile phase consisting of 85:15 (v/v) methanol: 20mM potassium dihydrogen phosphate buffer at a flow rate of 1.2 ml/min. Detection was performed at 227 nm. Metformin hydrochloride, Nateglinide, and Pioglitazone hydrochloride eluted with retention times of 2.15, 3.787, and 4.57 min, respectively. The method was meticulously validated following ICH guidelines, demonstrating specificity, rapidity, accuracy, precision, reliability, and reproducibility. Calibration curves were linear over concentration ranges of 50-250 mg/ml for Metformin hydrochloride, 3.0-15.0 mg/ml for Nateglinide, and 2-10 mg/ml for Pioglitazone hydrochloride. Limits of detection were determined to be 0.03, 0.22, and 0.008 mg/ml, and limits of quantification were found to be 0.09, 0.67, and 0.02 mg/ml for Metformin hydrochloride, Nateglinide, and Pioglitazone hydrochloride, respectively.(38)

16. T. T. Unade and A. K. Pawar (2022) the method employed a Lunna C18 column (100 mm x 2.6 mm, 1.6 µ) with trifluoroacetic acid buffer: acetonitrile (70:30, v/v) as the mobile phase at a flow rate of 1 ml/min and detection wavelength of 227 nm. Validation and forced degradation studies were conducted following ICH guidelines. Under optimized conditions, retention times

were 1.719 min and 2.845 min for Metformin and Gliclazide, respectively. The method exhibited excellent linearity from 25.0-375.0 µg/ml for Metformin and 4.0-60.0 µg/ml for Gliclazide, with coefficients of determination (r^2) exceeding 0.99. Detection limits were 0.25 µg/ml for Metformin and 0.04 µg/ml for Gliclazide, while recovery results ranged from 99.63% to 101.23%. Precision studies showed % RSDs of less than 1.11% for both drugs. The method effectively resolved analyte peaks from degrading peaks in forced degradation studies, confirming its stability-indicating capability. (39)

17. MousaSha'at and Adrian Florin Spac (2022) present a quality by design (QbD)-enabled approach for developing a high-performance liquid chromatographic (HPLC) method to estimate metformin hydrochloride (M-HCl) with precision and accuracy. Design of experiments(DoE) and risk assessment via Ishikawa diagram identified critical method parameters (CMPs), with buffer pH and mobile phase composition as key variables for mathematical modelling using a central composite design (CCD). The optimized conditions utilized 0.02 M acetate buffer pH = 3/methanol (70/30, v/v) at a flow rate of 1 mL/min on a Thermoscientific ODS HypersylTM column (250 × 4.6 mm, 5 µm) with UV detection at 235 nm and an oven temperature of 35 °C. Method robustness was evaluated by critical analytical attributes (CAAs) such as retention time, peak area, and symmetry factor, optimized simultaneously using the desirability function. (40)

18. John and Khan (2022) presented a comprehensive study on the simultaneous determination of metformin and teneligliptin, two vital antidiabetic medications, in tablet formulations. The method utilized a Thermo C18 analytical column (250 mm × 4.6 mm i. d., 5.0 µm) and employed an isocratic mobile phase of acetonitrile: water (80:20 v/v). The mobile phase was rigorously prepared by filtration and degassing to ensure purity and consistency. Chromatographic separation was achieved with a flow rate of 1.0 mL/min, and detection was performed at 228 nm. Retention times were reported as approximately 3.458 min for metformin and 5.125min for teneligliptin, with a total chromatographic analysis time per sample of around 20 minutes. Validation results indicated the method's robustness, demonstrating high recovery rates and low relative standard deviations, thereby confirming its applicability for routine quantitative analysis of metformin and teneligliptin in pharmaceutical formulations.(41)

19. Patel Khusbhu and Shah Ujashumar A (2023) developed a new stability indicating reverse phase liquid chromatography method using Quality by Design (QbD) principles for simultaneous determination of Linagliptin and Metformin HCl.The study employed a design of experiments and response surface methodology, specifically Centre Composite Design (CCD), to optimize the RP-HPLC conditions for effective separation and resolution, achieving a chromatographic resolution

of 6.4. Chromatographic separation was performed on a Water C18 column (250 mm × 4.5 mm × 5 µm) with a mobile phase consisting of acetonitrile and methanol (75:25 % v/v) at a flow rate of 1.0 mL/min. Detection of Linagliptin and Metformin HCl was conducted at 245 nm. The method exhibited linearity in the concentration ranges of 0.5-3 µg/mL for Linagliptin and 100-600 µg/mL for Metformin HCl, with recovery rates 98% to 102%. Stress testing demonstrated effective separation of degradation products from the analytes. Validation according to ICH guidelines confirmed the method's specificity, rapidity, precision, and robustness for routine analysis in pharmaceutical dosage forms, indicating its suitability for quality control applications.

20. Popat Mohite and Ramesh Bhusal (2024) developed and validate a reliable method for the simultaneous assessment of Metformin and Vildagliptinin bulk and pharmaceutical dosage forms. Metformin, an antidiabetic drug, inhibits hepatic glucose production, while Vildagliptin, a DPP-4 inhibitor, enhances insulin secretion and reduces blood sugar-elevating hormones, synergistically lowering blood glucose levels. The study utilized a Gs-Tek INNOWAX column (30 m length, 0.25 mm internal diameter, 1.8 µm film thickness) for chromatographic separation. Detection was achieved using a Flame Ionization Detector (FID). The optimized conditions included an injection volume of 1 µL in split mode (10:1 ratio), a carrier gas flow rate of 1 mL/minute of nitrogen, injector and detector temperatures set at 300 °C and 250 °C, respectively. The initial oven temperature of 100 °C was held for seven minutes and then ramped at 10 °C per minute until reaching 300 °C. The method development and validation were conducted according to standard guidelines, ensuring specificity, precision, and accuracy suitable for routine analysis in pharmaceutical laboratories.(42)

4. Importance of Proposed Work

1. Application of Quality by Design (QbD):

- ❖ Implemented a systematic QbD approach to enhance method development and ensure robust analytical performance.
- ❖ Identified and optimized Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs) to achieve a robust method.
- ❖ Utilized Design of Experiments (DoE) to understand the influence of various factors on method performance, leading to a well-defined method design space.

2. Development of the RP-HPLC Method:

- ❖ Developed a novel RP-HPLC method for the simultaneous estimation of anti-diabetic and cardiovascular drugs in bulk and formulations.
- ❖ Optimized of chromatographic conditions to achieve high resolution, sensitivity, and reproducibility.
- ❖ Developed reliable and reproducible method with a short run time, making it suitable for routine quality control.

3. Method Validation:

- ❖ Performed extensive validation of the RP-HPLC method according to ICH guidelines, ensuring specificity, linearity, accuracy, precision, robustness, LOD, and LOQ.
- ❖ Demonstrated the method's ability to accurately quantify the drugs in the presence of excipients and potential impurities, confirming its applicability for quality control.

4. Forced Degradation Studies:

- ❖ Performed comprehensive forced degradation studies under various stress conditions (acidic, basic, oxidative, thermal, and photolytic) to assess the stability of the selected drugs.
- ❖ Identified and characterized of degradation products contribute valuable information on the stability profile of the drugs.
- ❖ Developed the RP-HPLC method as a stability-indicating method capable of distinguishing the active drugs from their degradation products.

5. Regulatory Compliance and Industry Relevance:

- ❖ Ensured that the developed methods comply with regulatory requirements, making them suitable for use in the pharmaceutical industry.
- ❖ Provided a robust and validated analytical tool that can be employed in the routine analysis and quality control of antidiabetic drugs in both bulk and formulated products.
- ❖ Contributed to improving the quality and safety of anti-diabetic and cardiovascular medications by providing reliable methods for their analysis.

5. Aim and Objective

5.1 Aim:

To develop a robust and validated QbD, RP-HPLC method, and forced degradation studies for the quantitative estimation of anti-diabetic and cardiovascular drugs in bulk and formulations.

5.2 Objectives:

1. Develop a QbD-Based Analytical Method; Apply Quality by Design (QbD) principles to systematically develop and optimize an analytical method for the simultaneous estimation anti-diabetic and cardiovascular drugs in bulk and pharmaceutical formulations.

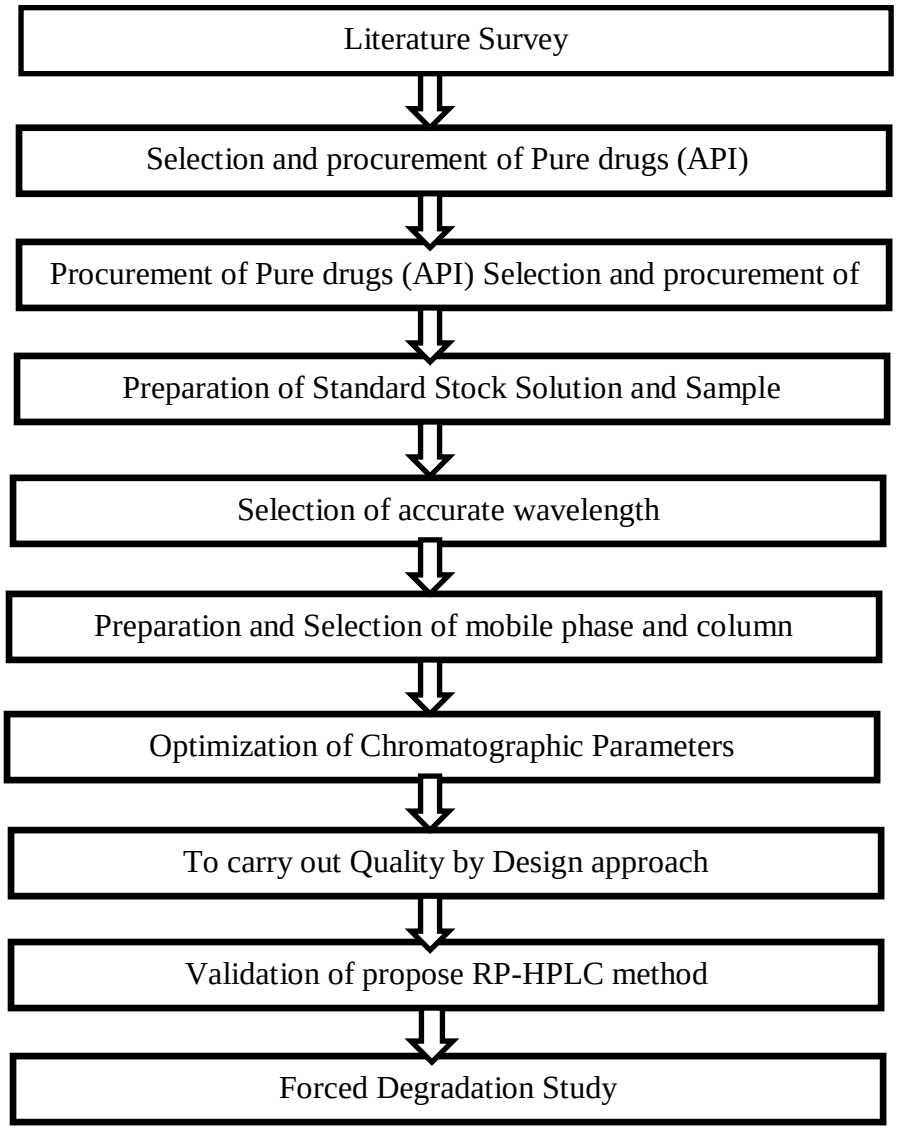
2. Optimize and Validate RP-HPLC Method; Develop, optimize, and validate a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method to ensure accurate, precise, and reproducible quantification of anti-diabetic and cardiovascular drugs

3. To conduct Forced Degradation Studies; Develop, optimize, and validate a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method to ensure accurate, precise, and reproducible quantification of anti-diabetic and cardiovascular drugs

4. Validate Analytical Methods According to the ICH Guidelines; Conduct comprehensive validation of the developed analytical methods in accordance with International Council for Harmonisation (ICH) guidelines, assessing parameters such as accuracy, precision, linearity, specificity, and robustness.

5. Apply Validated Methods to Real Pharmaceutical Formulations; validate the suitability of the validated analytical methods for routine quality control by analyzing real pharmaceutical formulations containing the triple combination of anti-diabetic and cardiovascular drugs.

6. Methodology


Literature Survey
Selection and procurement of Pure drugs (API)
Procurement of Pure drugs (API) Selection and procurement of
Preparation of Standard Stock Solution and Sample
Selection of accurate wavelength
Preparation and Selection of mobile phase and column
Optimization of Chromatographic Parameters
To carry out Quality by Design approach
Validation of propose RP-HPLC method
Forced Degradation Study

7. Research Work Plan:

Sr. No	Work	Time Period
1	Literature Survey	6 Months
2	Procurement of API and Excipient	2 Months
3	Selection of Solvent to be used	2 Months
4	Preparation of Standard Stock Solution and Sample Solution	1 Months
5	Selection of wavelength and Preparation & Selection of Mobile Phase	1 Months
6	Optimization of Chromatographic Conditions	3 Months
7	Implementation of Quality by Design Approach	6 Months
8	Validation of Propose RP-HPLC Method	6 Months
9	Forced Degradation Study	3 Months
10	Other Suggested Work and Compilation of Data	6 Months
	Total Duration of Work	36 Months

8. Expected Outcome of the Research

- The expected outcomes of this research include the development of robust and validated analytical methods for the simultaneous estimation of anti-diabetic and cardiovascular drugs in pharmaceutical formulations by applying Quality by Design (QbD) principles, the research aims to enhance method reliability and reduce variability, ensuring consistent and accurate results.
- Additionally, stability-indicating methods will be developed through Forced Degradation studies, which will ensure the accurate detection and quantification of degradation products.
- The developed methods will be validated according to International Council for Harmonization (ICH) guidelines, ensuring compliance with regulatory requirements.
- Finally, the practical applicability of these methods will be demonstrated for routine quality control and stability testing of pharmaceutical formulations, confirming their suitability for use in the pharmaceutical industry.

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